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GUAIAN-5,12-OLIDES FROM *LEONTODON HISPIDUS*

CHRISTIAN ZIDORN, ERNST P. ELLMERER-MÜLLER† and HERMANN STUPPNER*

Institut für Pharmakognosie der Universität Innsbruck, Josef-Möller-Haus, Innrain 52, A-6020 Innsbruck, Austria; † Institut für Organische Chemie der Universität Innsbruck, Innrain 52a, A-6020 Innsbruck, Austria

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Key Word Index—*Leontodon hispidus*; Asteraceae; Lactuceae; sesquiterpene lactones; guaianolides.

Abstract—The dichloromethane extract of the whole plant of *Leontodon hispidus* afforded one new and two known guaian-5,12-olides. The structure of the new compound has been established by extensive 2D NMR techniques as 14-hydroxyhypocretenolide-β-D-glucopyranoside-4′-14″-hydroxyhypocretenoate.

INTRODUCTION

Leontodon hispidus L. distributed over most of Europe to Asia Minor, the Caucasus and Persia is one of about 50 species of the genus *Leontodon* [1]. Only for one species, *L. autumnalis* L., has the occurrence of sesquiterpene lactones (jacquilenin and 8-deoxylactucin) been reported [2]. Guaian-5,12-olides, a rare group of sesquiterpene lactones, have so far only been found in three other members of the Lactuceae tribe of the Asteraceae: *Hypochoeris cretensis* (L.) Bory & Chaub., *Hedypnois cretica* (L.) Dum.-Courset and *Crepis aurea* (L.) Cass. [3–5].

RESULTS AND DISCUSSION

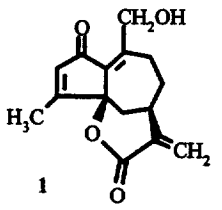
The CH_2Cl_2 extract of the air dried whole plant of *L. hispidus* was repeatedly chromatographed on Sephadex LH-20, silica gel and reversed phase material to give one new hypocretenolide derivative (**3**) in addition to the known compounds 14-hydroxyhypocretenolide (**1**) and 14-hydroxyhypocretenolide-β-D-glucopyranoside (**2**).

Identification of **1** and **2** was based on a comparison of their MS (positive ESI *m/z* 261 $[M+H]^+$ and 423 $[M+H]^+$, respectively) and ^1H NMR data with those given in literature [4]. Since the ^{13}C NMR data from **1** and **2** have not yet been published these are shown in Table 1.

The molecular formula of **3** was determined to be $C_{36}H_{42}O_{12}$ from the HR FAB mass spectrum (*m/z* 683.2654 $[M+H]^+$). The ESI mass spectrum showed a quasimolecular ion peak at *m/z* 683 $[M+H]^+$ and major fragments at *m/z* 423 $[M-260+H]^+$ and 261 $[M-422+H]^+$ suggesting substance **3** to be a derivative of **2** esterified with the parent sesquiterpenoid acid of **1**.

This was confirmed by the ^1H NMR spectrum (Table 2) which showed signals for two olefinic methylene groups [δ_H 6.65 (*br d*, $J_{13,7}$ = 1.5 Hz) and δ_H 5.92 (*br s*) as well as δ_H 6.30 (*br d*, $J_{13,7}$ = 1.5 Hz) and δ_H 5.78 (*br s*)], two olefinic methyl groups (δ_H 2.16; *d*, $J_{15,3}$ = 1.5 Hz), two vinylic protons located in the α-position to a carbonyl function (δ_H 6.31; *q*, $J_{3,15}$ = 1.5 Hz and 6.03; *q*, $J_{3'',15''}$ = 1.5 Hz), two oxygen-bearing methylene groups (δ_H 5.30 and 4.98; *d*, *J* = 13.5 Hz; δ_H 4.97 and 4.65; *d*, *J* = 14.5 Hz) and one anomeric sugar proton (δ_H 4.45; *d*, *J* = 8.0 Hz). The glucose moiety was identified by a combination of selective HSQC, HSQC-TOCSY and phase sensitive COSY experiments. The ^{13}C NMR spectrum showed the presence of 36 carbons: two methyls, 11 methylenes, nine methines and 14 quarternaries. Fifteen carbon signals assignable to the sesquiterpene lactone groups of **3** were almost superimposable on the corresponding signals of **2**, whereas those of the sesquiterpene group with the opened γ-lactone ring showed the following shift differences: the signals of C-4″ (δ 177.7), C-6″ (δ 40.4) and C-11″ (δ 146.5) were shifted downfield by 7.1, 7.8 and 8.2 ppm, respectively, and those of C-5″ (δ 79.9), C-8″ (δ 28.8) and C-13″ (δ 124.9) shifted upfield by 10.0, 5.6 and 6.0 ppm (Table 2). In comparison with the ^{13}C NMR data of **2** the C-4′ (δ 72.7) signal of the glucose moiety was shifted to highfield (+1.2 ppm) and the resonances of C-3′ (δ 75.7) and C-5′ (δ 75.3) were shifted downfield (−2.6 and −3.0 ppm, respectively) indicating acylation of the

*Author to whom correspondence should be addressed. Fax: 0043-512-507-2939; Email: Hermann.Stuppner@uibk.ac.at



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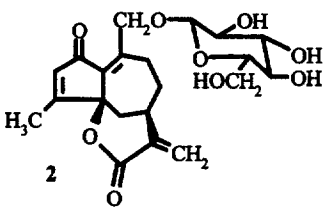

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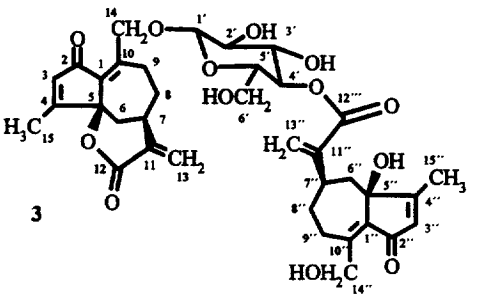

3

Table 1. ^{1}H NMR data of compound **3** (500 MHz, δ-values in MeOH-d_4)‡

	H		H
3	6.31 1H, *q* (1.5)	3″	6.03 1H, *q* (1.5)
6	2.55 1H, *ddd* (14.5, 4.5, 1.0)	6″	2.04 1H, *d* (11.0)
	2.16 1H,†		2.01 1H, *d* (11.0)
7	3.39 1H, *ddd* (4.5, 3.0, 1.5)	7″	2.64 1H,†
8	2.64 1H, *dddd* (15.0, 13.0, 3.0, 1.0)	8″	2.25 1H, *m*
	2.10 1H,†		1.83 1H, *m*
9	2.90 1H, *ddd* (16.5, 5.5, 2.0)	9″	3.01 1H, *ddd* (16.0, 7.5, 7.5)
	2.49 1H, *ddd* (16.5, 13.0, 2.0)		2.76 1H, *ddd* (16.0, 7.0, 5.0)
13	6.65 1H, *br d* (1.5)	13″	6.30 1H, *br d* (1.5)
	5.92 1H, *br s*		5.78 1H, *br s*
14	5.30 1H, *d* (13.5)	14″	4.97 1H, *d* (14.5)
	4.98 1H, *d* (13.5)		4.65 1H, *d* (14.5)
15	2.16 3H, *d* (1.5)	15″	2.16 3H, *d* (1.5)
1′	4.45 1H, *d* (8.0)		
2′	3.36 1H, *dd* (9.5, 8.0)		
3′	3.64 1H, *dd* (9.5, 9.5)		
4′	4.90 1H, *dd* (9.5, 9.5)		
5′	3.52 1H, *ddd* (9.5, 5.5, 2.0)		
6′	3.64 1H, *dd* (12.0, 2.0)		
	3.56 1H, *dd* (12.0, 5.5)		

† Signals are overlapping.
‡ Coupling constants (J) in Hz are given in parentheses.
§ All assignments are based on HMBC and HSQC experiments.

hydroxyl group at C-4′ [8]. This was verified by a HMBC experiment (Table 3) which showed long range couplings between H-4′ of the sugar moiety and the carbonyl carbon 12″ of the sesquiterpenoid acid part of the molecule. Correlations; between the geminal protons of C-14 and C-1′ as well as the anomeric proton H-1′ and C-14 confirmed the linkage between the glucose and the 14-hydroxyhypocretenolide moiety. β-Glucosidation was proven by the characteristic coupling of the anomeric proton at C-1′ ($J_{1',2'} = 8.0$ Hz). According to Bohlmann and Singh [3] the 5,12-guaianolide ring requires an equatorial orientation of H-7, which is in agreement with the observed coupling between H-7 (δ_H 3.39; *ddd*) and H-13 (δ_H 6.65; *d*, $J_{7,13} = 1.5$ Hz). The configuration at C-7″ has been proven to be identical with that at C-7, since **3** in a mixture of water and methanol (3:2) decomposes into equimolar amounts of **1** and **2**. This

Table 2. ^{13}C NMR data of compounds **1–3** (75.1 MHz, δ values in MeOH-d_4)

C	1	2	3	C	3
1	137.1 *s*	136.4	137.5	1″	136.2
2	195.2 *s*	194.7	194.7	2″	197.6
3	136.0 *d*	134.7	135.6	3″	131.8
4	171.5 *s*	170.6	171.1	4″	177.7
5	91.0 *s*	89.9	90.7	5″	79.9
6	33.4 *t*	32.6	32.7	6″	40.4
7	39.0 *d*	38.2	38.6	7″	37.3
8	35.1 *t*	34.5	34.4	8″	28.8*
9	26.0 *t*	25.7	25.8	9″	28.9*
10	157.9 *s*	154.1	154.6	10″	153.4
11	138.9 *s*	138.3	138.7	11″	146.5
12	167.3 *s*	165.9	166.9	12″	167.8
13	131.0 *t*	130.9	130.5	13″	124.9
14	61.5 *t*	68.3	68.3	14″	61.9
15	13.0 *q*	11.9	12.7	15″	12.9
1′		104.5 *d*	104.7		
2′		74.5 *d*	76.0		
3′		78.3 *d*	75.7		
4′		71.5 *d*	72.7		
5′		78.3 *d*	75.3		
6′		61.8 *t*	62.4		

* Signals may be interchangeable.

† Assignments are based on DEPT, HMBC and HSQC experiments.

Table 3. HMBC data for compound **3**

H	C	H	C
3	1, 2, 4, 5, 15	3″	1″, 2″, 4″, 5″, 15″
6	1, 4, 5	6″	1′, 5″, 8″
	4, 5		1′, 5″, 8″
7	—	7″	—
8	10	8″	7″, 9″, 10″
	7		7″, 9″
9	1, 7, 8, 10	9″	1″, 7″, 8″, 10″, 14″
	1, 7, 8, 10, 14		1″, 7″, 8″, 10″, 14″
13	7, 11, 12	13″	7″, 11″, 12″
	7, 12		7″, 12″
14	1, 9, 10, 1′	14″	1″, 9″, 10″
	1, 9, 10, 1′	14*″	1″, 9″, 10″
15	3, 4, 5	15″	3″, 4″, 5″
1′	14		
2′	1′, 4′		
3′	2′, 5′		
4′	2′, 6′		
5′	3′, 4′		
6′	5′		
	5′		

reaction could be inhibited by using pure MeOH as solvent or by acidifying aqueous MeOH with TFA. Thus, **3** is 14-hydroxyhypocretenolide-β-D-glucopyranoside-4′-14″-hydroxyhypocretenoate.

EXPERIMENTAL

Plant material

Leontodon hispidus L. was collected in June 1996 in the vicinity of Innsbruck. A voucher specimen is deposited at the Institute of Pharmacognosy.

Extraction and isolation of compounds **1–3**

Whole, air dried plants (3.1 kg) were ground and extracted exhaustively at room temp. with CH_2Cl_2 yielding 50 g of residue after evaporation of the solvent *in vacuo*. Fifteen grams of the residue were chromatographed on a Sephadex LH-20 column using MeOH as solvent. Frs containing compounds **1–3** were combined and repeatedly chromatographed on silica gel columns using gradients of EtOAc–MeOH. Final purification of compounds **1–3** was carried out by MPLC on a RP-18-column (Merck) using a gradient of TFA (0.01%)—MeOH–MeCN (3:1) to give **1** (8 mg), **2** (5 mg) and **3** (35 mg).

Decomposition of **3**

One mg amounts of **3** were dissolved respectively in 1.0 ml H_2O–MeOH (3:2), pure MeOH and H_2O–MeOH (3:2) acidified with TFA (pH 1.5) and kept at room temp. for 24 h. The solns were analyzed by HPLC using a Zorbax Rx-C18 4.6 mm × 25 cm column (P.N. 880967.902), a gradient of H_2O–MeOH starting from 4:1 to 39:61 in 24 min; flow rate: 1.0 ml min^{-1}; detection: 245 nm. R_ts of **1–3**: 13.4, 10.6 and 19.4 min, respectively.

Compound **3**. Amorphous wax-like substance melting between 96° and 102°. FAB-MS (positive ion), (rel. int.): *m/z* 683.2654 $[M+H]^+$ (100); ESIMS (positive ion), (rel. int.): *m/z* 705.5 $[M+Na]^+$ (14), 683.5 $[M+Na]^+$ (18), 665.5 $[M-H_2O+H]^+$ (23), 423.3 [M–sesquiterpenic acid + H]$^+$ (27), 405.2 [M–sesquiterpenoid acid–H_2O + H]$^+$ (62), 261.2 [M–sesquiterpenoid acid–glucose + H]$^+$ (100), 243.2 [M–sesquiterpenoid acid–glucose–H_2O + H]$^+$] (39).

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REFERENCES

1. Wagenitz, G., *Hegi Flora von Mitteleuropa*, Vol. VI/4 2nd Edn. Parey, Berlin, Hamburg, 1987, p. 1483.
2. Pyrek, J., *Phytochemistry*, 1985, **24**, 186.
3. Bohlmann, F. and Singh, P., *Phytochemistry*, 1982, **21**, 2119.

4. Harraz, F. M., Kassem, F. F., Grenz, M., Jakupovic, J. and Bohlmann, F., *Phytochemistry*, 1988, **27**, 1866.
5. Kisiel, W., *Fitoterapia*, 1994, **65**, 381.
6. Bohlmann, F., Jakupovic, J., Abraham, W.-R. and Zdero, Ch., *Phytochemistry*, 1981, **20**, 2371.
7. Bohlmann, F., Gupta, R. K. and Jakupovic, J., *Phytochemistry*, 1982, **21**, 460.
8. Jansson, P.-E., Kenne, L. and Schweda, E., *J. Chem. Soc. Perkin Trans. I*, 1987, 377.